Large-scale use of freeze-dried smallpox vaccine prepared in primary cultures of rabbit kidney cells

A. C. HEKKER,[1] J. M. BOS,[2] N. KUMARA RAI,[3]
J. KEJA,[4] G. CUBONI,[4] B. EMMET,[4] & J. DJALINS [5]

A lyophilized smallpox vaccine made from infected monolayer cultures of primary rabbit kidney cells was used together with a calf lymph vaccine in a field trial in Lombok, Indonesia, in 1973. About 60 000 children below 15 years of age were vaccinated: some 50 000 with the tissue culture vaccine and about 10 000 with calf lymph vaccine. Similar results were obtained with both vaccines in primary vaccinees and in revaccinees as regards the take rate, pock reactions, and serious secondary reactions.

Smallpox vaccines prepared on the skin of living animals have been used for the vaccination of human beings for many decades. These vaccines have been shown to be immunogenic as well as to protect against smallpox. Although the introduction of the seed virus principle, the use of a better defined strain of vaccinia virus, and, in many places, the use of freeze-dried vaccine instead of liquid glycerinated vaccine improved safety and stability, certain recent developments in virology have not yet been applied to the production of smallpox vaccine. Hitherto the results of the use of vaccines produced in cell cultures have been unsatisfactory, since passaging the vaccinia virus through cell cultures only a few times results in a vaccine that is less immunogenic. For this reason WHO does not accept vaccines made in tissue cultures if there have been more than 5 passages of the vaccinia virus (*8*).

In the Netherlands a freeze-dried smallpox vaccine was prepared in primary rabbit kidney cell cultures (*5*). The seed virus for the production of this vaccine was prepared in calves and was the same as that used for the production of calf lymph vaccine. The virus was thus not more than one passage removed from animal skin. Satisfactory potency was obtained by concentration through ultrafiltration.

Intensive laboratory and field studies with this vaccine have been conducted in the Netherlands. No differences as regards the development of vaccination illness, the take rate, and antibody-inducing capacity were observed between the tissue culture vaccine and calf lymph vaccine in approximately 1600 persons subjected to either primary vaccination or revaccination (*4*, *6*). In order to supplement and confirm these results on a larger scale under field conditions, the island of Lombok, Indonesia, was chosen for the mass vaccination of 50 000 children below 15 years of age with the tissue culture vaccine. The take rate and adverse reactions were observed. For comparative purposes, 10 000 other children were vaccinated with calf lymph vaccine. Both vaccines were produced in the Rijks Instituut voor de Volksgezondheid, Bilthoven, were freeze-dried, and had a log potency of 8.5 pock-forming units per millilitre. The studies were conducted in May and June 1973. The Indonesian government authorities were involved in the discussions about the study from the beginning and fully agreed to it. The results are presented in this report.

LOCATION, MATERIALS, AND METHODS

Lombok

Lombok is situated east of Java, between Bali and Sumbawa. The island has an area of approximately 5000 km². The northern half is mountainous and the southern half flat. The great majority of the about 1.5 million inhabitants live in a strip 15–20 km wide,

[1] Head, Laboratory of Virology (formerly Head, Smallpox Vaccine Laboratory), Rijks Instituut voor de Volksgezondheid, Postbus 1, Bilthoven, Netherlands.

[2] Assistant virologist, Smallpox Vaccine Laboratory, Rijks Instituut voor de Volksgezondheid, Bilthoven.

[3] Directorate General, Department of Health, P.O. Box 223, Jakarta, Indonesia.

[4] World Health Organization, P.O. Box 302, Jakarta, Indonesia.

[5] Directorate of the Health Service, Propinsi Nusa Tenggara Barat, Mataram, Lombok, Indonesia.

which traverses the island from west to east just below the centre. There are five towns in this strip: Ampenan, Mataram (capital of the island and of the west district, Lombok Barat), Cakranegara, Selong (capital of the east district, Lombok Timur), and Praja (capital of the central district, Lombok Tengah). The total population of these five towns is approximately 100 000. The other 1.4 million people live mostly in small villages scattered over the island. The people live on agricultural products, mainly rice.

Design of the study

On the basis of a scar survey, carried out by the Provincial Health Services in July 1972 on 7000 children, at least 20% (12 000) primary vaccinees could be expected in a sample of 60 000 children. This number was considered to be sufficient for the study. Fifteen experienced vaccinators were available for 8 weeks for vaccination and follow-up by house-to-house visit of some 60 000 children below 15 years of age. The tissue culture smallpox vaccine was prepared from the Elstree strain as described earlier (*5*). The calf lymph vaccine was prepared in the same laboratory by a technique similar to that described in " Methodology of freeze-dried smallpox vaccine production ".[a] Both types of vaccine were freeze-dried in 0.2-ml quantities. The titrations were done as described by Hekker & Bos.[b] The log titre of both vaccines was 8.5 pock-forming units per millilitre. During the trial and at the end, vaccine samples were taken in the field and titrated in the laboratory in Bilthoven. The virus titre was the same as had been found before. For practical purposes it was decided to vaccinate on 5 days a week with tissue culture vaccine and on 1 day a week with calf lymph vaccine.

Preparatory phase

After the central and local government authorities had agreed to the proposed study, the local staff, vaccinators, and recorders were selected and instructed. Out of a total of 206 villages and 5 towns, 55 villages and 4 towns were randomly chosen for inclusion in the study. The cooperation of the village heads and village population was sought and obtained. Forms were prepared to record the family name, first name, age, sex, and vaccination history. A schedule was made for the vaccination and follow-up of 60 000 children with the tissue culture vaccine and 10 000 with the calf lymph vaccine within 6–8 weeks.

Vaccination

The vaccinations were carried out by 15 teams during May and June 1973. Each team consisted of one vaccinator, one recorder, and one inhabitant of the village where the team was working. An average of 1700 vaccinations were performed each day with a bifurcated needle. Four medical officers and one technical officer assisted in the instruction, organization, coordination, and supervision. Two Landrovers and three jeeps were available for transport.

Readings

For the reading of vaccination reactions the vaccinees were revisited 13–14 days after vaccination. For this purpose 3 or 4 teams were used. The teams were instructed to consider a vaccination result as positive only if the child showed a so-called " major reaction " as described in the " Handbook for smallpox eradication programmes in endemic areas ".[c] If the children were absent on the day of the inspection, further attempts were made to identify the primary vaccinees and to record the results of vaccination at a second visit within a couple of days of the first. An extra form was designed to obtain as much information as possible about the vaccinees who died during the 13–14 days following vaccination.

RESULTS

A total of 61 808 children were vaccinated: 51 268 (82.9%) with tissue culture vaccine and 10 540 (17.1%) with calf lymph vaccine; 15 390 (30.0%) of those vaccinated with tissue culture vaccine and 2992 (28.4%) of those vaccinated with calf lymph vaccine were primary vaccinees. The vaccinated children were distributed into 4 age groups: <1, 1–4, 5–6, and 7–14 years of age. The last two age groups were fixed because, during 1965, 1966, and early 1967 Lombok suffered a smallpox epidemic of some 21 000 cases. Children of 7 years and over had therefore lived through this epidemic.

Comparison of the two vaccine groups

Sex distribution. Since no sex differences were found, the results for boys and girls are presented together.

[a] Unpublished WHO document SE/68.3, Rev.1, 1968.

[b] Hekker, A. C. & Bos, J. M. Potency testing of smallpox vaccine. Unpublished WHO document SE/72.43, 1972.

[c] Unpublished WHO document SE67/5 Rev.1, 1967.

Table 1. Distribution by age group of children subjected to primary vaccination and revaccination with tissue culture or calf lymph vaccine

Age group (years)	Primary vaccination				Revaccination			
	Tissue culture vaccine		Calf lymph vaccine		Tissue culture vaccine		Calf lymph vaccine	
	No.	%	No.	%	No.	%	No.	%
< 1	4 014	26.1	729	24.4	201	0.6	51	0.7
1–4	9 572	62.2	1 890	63.2	14 696	41.0	3 036	40.2
5–6	1 180	7.7	251	8.4	9 192	25.6	1 940	25.7
7–14	624	4.1	122	4.1	11 789	32.9	2 521	33.4
Total	15 390	100	2 992	100	35 878	100	7 548	100

Age group distribution. Table 1 gives the distribution according to age group of children who received primary vaccination and revaccination with each of the two vaccines. For both vaccines, the distribution of primary vaccinees was not significantly different from that of revaccinees ($P > 0.10$).

Unrecorded vaccination results. Table 2 gives the numbers and percentages of unrecorded results of primary vaccination and revaccination with each of the two vaccines and in each of the four age groups. The percentages of unrecorded results varied somewhat among the different age groups and the percentages for calf lymph vaccine were somewhat higher than those for tissue culture vaccine. This can be explained by the fact that absenteeism in different villages varied between 0% and 35% depending on the presence of a school, a market, or work in the rice fields. Calf lymph vaccine was used in only 15 of the 55 villages in which children were vaccinated, and in 3 of these 15 villages extremely high percentages of absenteeism were found.

Success rate. Table 3 shows the success rates for primary vaccination and revaccination according to type of vaccine for each of the four age groups. There were no significant differences in the success rate between the two types of vaccine, either after primary vaccination or after revaccination in the corresponding age groups. The success rate in the age group 7–14 years in primary vaccinees and in revaccinees for both types of vaccine was a little lower

Table 2. Number and proportion (%) of unrecorded results of primary vaccination and revaccination with each of the two vaccines in each of the four age groups

Age group (years)	Primary vaccination				Revaccination			
	Tissue culture vaccine		Calf lymph vaccine		Tissue culture vaccine		Calf lymph vaccine	
	No.	%	No.	%	No.	%	No.	%
< 1	120	3.0	34	4.7	12	6.0		8/51 [a]
1–4	436	4.6	111	5.9	1 286	8.8	343	11.3
5–6	72	6.1	22	8.8	1 102	12.0	292	15.1
7–14	85	13.6	29	23.8	2 712	23.0	640	25.4
Total	713	4.6	196	6.6	5 112	14.2	1 283	17.0

[a] Numerator = number of unrecorded vaccination results; denominator = number of vaccinees in the group.

Table 3. Success rate for primary vaccination and revaccination with each of the two vaccines in each of the four age groups

Age group (years)	Primary vaccination				Revaccination			
	Tissue culture vaccine		Calf lymph vaccine		Tissue culture vaccine		Calf lymph vaccine	
	No. in group recorded	Success rate (%)	No. in group recorded	Success rate (%)	No. in group recorded	Success rate (%)	No. in group recorded	Success rate (%)
< 1	3 894	96.8	695	96.4	189	73.0		26/43 [a]
1–4	9 136	97.8	1 779	98.0	13 410	78.6	2 693	75.8
5–6	1 108	96.6	229	93.5	8 090	77.4	1 648	74.9
7–14	539	88.1	93	88.2	9 077	65.8	1 881	61.7
Total	14 677	97.1	2 796	96.9	30 766	74.7	6 265	71.2

[a] Numerator = number of successful vaccinations; denominator = number of vaccinees in the group.

than the success rate in the other age groups for the corresponding type of vaccine.

Complications after vaccination. All reported illnesses that occurred subsequent to vaccination were carefully evaluated. None of the children in either group experienced disseminated vaccinia, eczema vaccinatum, or vaccinia necrosum. Although transient allergic skin reactions may have occurred, none was serious enough to be brought to the attention of the investigators. A number of cases of severe malaria, gastroenteritis, and pneumonia occurred in both vaccinated and unvaccinated children. Among those in the study group, illnesses were randomly distributed according to the time of onset and their occurrence appeared to bear no relationship to vaccination.

One case of encephalitis occurred that may have been vaccine-related. The patient—a 5-month-old girl—underwent primary vaccination with tissue culture vaccine on 7 May, the first day of the campaign. One week later the mother brought the child to the doctor because it had fever. The child had a major reaction at the vaccination site. A nurse injected penicillin and streptomycin intramuscularly and advised the mother to come back if the fever did not disappear. Four days later the mother returned. The fever had persisted and the child had experienced convulsions and had been unconscious for several days. The body temperature was 41°C and the child showed neck stiffness. The corneal reflex was negative. Late at night barbiturate and antipyretic injections were given. The next morning the body temperature was 38.5°C and the neck stiffness had disappeared, but the child was still unconscious. A blood smear showed no malaria parasites. The same morning the child died from asphyxia during a convulsion.

DISCUSSION

Smallpox vaccine made in tissue culture has been used on a very limited scale. This may be due to difficulties in preparing a vaccine that is satisfactory in potency and stability. Ehrengut (*2*) mentions the long-term use of smallpox vaccine made in monolayer cultures of primary bovine embryonic muscle cells. The vaccinia virus in this vaccine was passaged 10 times in cultures of these cells. In order to fulfil the WHO requirements (*8*), the number of passages was reduced to five. However, in order to obtain, in primary vaccinees and in revaccinees, a take rate comparable to that obtained with dermovaccine, the titre of the tissue culture vaccine had to be at least 0.5 logs higher than the titre of the dermovaccine.

Undoubtedly, in the present trial, some who had faint or imperceptible vaccination scars were erroneously assigned to the unvaccinated group. Since smallpox vaccination scars become less evident in time, such errors of assignment may be expected to be more frequent with older than with younger children. Moreover, it may be assumed [a] that about

[a] Foster S. Persistence of facial scars of smallpox in West African populations. Unpublished WHO document SE/72.34, 1972.

one-third of those who had experienced smallpox during the 1965-1966 smallpox epidemic in Lombok, no longer bore the destructive facial pockmarks and so were erroneously assigned to the unvaccinated group. Others who were already protected against clinical illness by prior vaccination, undoubtedly experienced reinforcement of their immunity, thus accounting for a lower proportion of major reactions among revaccinees in the older age groups.

The patient with the symptoms described on page 282 apparently had encephalitis. The disease started to develop 6–7 days after primary vaccination. Approximately 90% of cases of post-vaccination encephalitis start to develop in the second week after vaccination (*1*). However, it is impossible to rule out with certainty the possibility that this child had post-vaccination encephalitis. In Indonesia and other tropical areas, there are various causes of encephalitis, especially in very young children. Even if this case was vaccine-related, post-vaccination encephalitis is a recognized complication of smallpox vaccination and occurs with a certain frequency (*2*, *3*, *7*). The incidence of encephalitis in mass vaccination campaigns—which include primary vaccination and revaccination at any age—varies between 1 in 8300 or less and 1 in 150 000 or more (*1*).

The conclusion is that lyophilized smallpox vaccine prepared from the Elstree strain, with only one passage in monolayer cultures of primary rabbit kidney cells and meeting the WHO requirements for safety, potency, and stability, is comparable with calf lymph vaccine as regards the success rate, pock reaction, and serious secondary reactions.

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RÉSUMÉ

UTILISATION À GRANDE ÉCHELLE DE VACCIN ANTIVARIOLIQUE LYOPHILISÉ, PRÉPARÉ EN PRIMOCULTURE DE CELLULES RÉNALES DE LAPIN

Pendant de nombreuses décennies, on a utilisé pour vacciner l'homme des vaccins antivarioliques préparés sur la peau d'animaux vivants, et ces vaccins se sont montrés immunogènes de même que protecteurs. Bien que l'introduction du principe du virus de semence, l'utilisation de souches de virus vaccinal mieux définies et, en maints endroits, l'emploi de vaccin lyophilisé à la place du vaccin liquide glycériné aient accru la sécurité et la stabilité, certains progrès récents de la virologie n'ont pas encore été appliqués à la production du vaccin antivariolique. Jusqu'ici les résultats obtenus avec des vaccins produits en culture de cellules n'ont pas été satisfaisants car un petit nombre de passages du virus vaccinal en culture cellulaire suffit pour abaisser le pouvoir immunogène du vaccin. C'est pourquoi l'OMS n'accepte pas ce type de vaccin si le nombre de passages du virus dépasse cinq.

Aux Pays-Bas, un vaccin antivariolique lyophilisé a été préparé en primoculture de cellules rénales de lapin. Le virus de semence utilisé pour la production de ce vaccin a été préparé sur des veaux, et il était identique à celui qui est utilisé pour la production du vaccin constitué de lymphe de veaux. Ainsi le virus vaccinal n'avait pas subi plus d'un passage à partir de son prélèvement de la peau de l'animal. Une activité satisfaisante a été obtenue grâce à la concentration par ultrafiltration. Ce vaccin a été soumis à des études intensives au laboratoire et en pratique, aux Pays-Bas, en mai et juin 1973. Chez environ 1600 enfants soumis à la primovaccination ou à une revaccination, on n'a observé aucune différence entre le vaccin classique et le vaccin préparé en culture de tissu au point de vue de l'apparition de la maladie vaccinale, du taux de « prises » et de la capacité de susciter des anticorps. Afin de compléter et confirmer ces résultats sur une plus grande échelle dans les conditions pratiques, on a choisi d'appliquer le vaccin de culture de tissu pour une vaccination de masse, dans l'île de Lombok, Indonésie, sur environ 50 000 enfants de moins de 15 ans. On a surveillé les taux de « prises » et les réactions adverses; il y a eu un cas d'encéphalite susceptible d'être en rapport avec le vaccin. A des fins de comparaison, environ 10 000 enfants ont été vaccinés

avec de la lymphe vaccinale de veau. Les deux vaccins ont été produits au Rijks Instituut voor de Volksgezondheid, Bilthoven, ont été lyophilisés et avaient une activité (log) de 8,5 unités de formation de pustules par millilitre. Des résultats similaires ont été obtenus avec les deux vaccins chez les primovaccinés et les revaccinés, du point de vue du taux de « prises », de la réaction pustuleuse et des réactions secondaires graves.

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